

Bio-produced Propionic Acid: A Review

Aladár Vidra¹, Áron Németh^{1*}

Received 27 March 2017; accepted after revision 19 June 2017

Abstract

Propionic acid is a platform chemical, antifungal agent and important chemical intermediate. Current industrial production of propionic acid is mainly through petrochemical processes because the conventional method of the propionic acid fermentation is uneconomical due to low product yield, productivity and product concentration caused by end-product inhibition. The coproduction of acetic and succinic acids in the propionic acid fermentation processes also makes downstream processing more complicated and costly. To the best of our knowledge there are several and recent reviews in the available literature on propionic acid fermentation processes and strain improvement techniques, but only a few on product recovery and purification, i.e. downstreaming. However, to realize a biorefinery, where propionic acid is a key intermediate, complex discussion of up-, and downstreaming is required. Therefore in this review a short overview of the whole bio-based propionic acid production process is presented including recent results of both upstream and downstream area. Thus the biosynthetic pathways, the significant results of native and recombinant producer strains as well as product recovery are discussed.

Keywords

propionic acid, propionic acid pathway, yield, productivity, titre, recovery

1 Introduction

Propionic acid is a valuable C-3 platform chemical. Accordingly, propionic acid and its derivatives are used in agriculture, food and pharmaceutical industries, e.g. propionic acid is an important chemical intermediate in the synthesis of herbicides, perfumes, cellulose fibres and pharmaceuticals. As a three-carbon building block, it is used as a precursor for high-volume commodity chemicals such as propylene [1]. Propionic acid and its calcium, sodium and potassium salts are widely used as preservatives in animal feed and human food. Some of them are also important mould inhibitors [2]. Currently, propionic acid is mainly produced by petrochemical routes, i.e., the Reppe, Larson and Fischer-Tropsch processes. Biotechnological production of propionic acid from renewable bioresources has attracted increasing interest in terms of research because of the problems associated with increased oil prices and the benefits of eco-friendly production [3]. However, bio-based propionic acid is more expensive than that synthesized from chemical routes because of the low propionic acid concentration and levels of productivity caused partly by product inhibition. The produced by-products, e.g. acetic and succinic acids, make downstream processing more complicated and costly in terms of product recovery and purification [4]. The global propionic acid market stood at around 400.0 kilotons in 2014, only a minor proportion of that was produced biologically [5].

2 Producing pathways

Three different biochemical pathways are known for the production of propionic acid: the succinate pathway, the acrylate pathway, and the propanediol pathway [6, 7].

2.1 Succinate pathway

The succinate pathway (Fig. 1), alias dicarboxylic acid pathway follows glycolysis which converts glucose and glycerol into pyruvate. Pyruvate is converted into oxaloacetate (OAA) by OAA transcarboxylase (EC 6.4.1.1) and propionate is generated through several intermediates, including malate, succinyl-CoA, and propionyl-CoA [8]. There are two key enzymes which catalyze multiple reactions. One of them is oxaloacetate

¹ Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, H-1111 Budapest, Budafoki út 6-8., Hungary

* Corresponding author, e-mail: naron@f-labor.mkt.bme.hu

transcarboxylase (EC 6.4.1.1) which transfers the carboxyl group from methylmalonyl-CoA to pyruvate with the simultaneous formation of oxaloacetate and propionyl-CoA. The other key enzyme is CoA transferase (EC 2.8.3.5) which reversibly transfers the CoA part from propionyl-CoA to succinate to form succinyl-CoA together with the end product propionate. In the succinate pathway acetate is a byproduct which is generated from pyruvate when the pyruvate dehydrogenase complex (EC 1.2.4.1) decarboxylates pyruvate to form acetyl-CoA, which is then converted into acetate by phosphotransacetylase (EC 2.3.1.222) and acetate kinase (EC 2.7.2.1) [9]. In some cases, like in *Propionibacterium acidipropionici* acetyl-CoA is converted into acetate by acetate-CoA ligase (EC 6.2.1.13) [10].

Bacteroidetes, several *Firmicutes* and some Gram-negative bacteria use the succinate pathway via methylmalonyl-CoA for propionate production. While *Bacteroidetes* mainly utilize polysaccharides and peptides as substrates of this metabolic pathway, strains belonging to *Firmicutes* use organic acids to produce propionate [11, 12]. Some Gram-negative bacteria exhibit special propionate metabolic activity together with the application of the succinate pathway, which provide a special degree of potential for them. *Phascolarctobacterium succinatutens* can grow on succinate alone whereas *Veillonella parvula* gains additional energy from succinate in the presence of lactate as the main growth substrate. *Selenomonas ruminantium* strains utilize carbohydrates to produce lactate, acetate and propionate and some are also able to use lactate for growth [6]. *Pelobacter propionicus* can consume alcohol to form propionate [13].

2.2 Acrylate pathway

The second propionate forming pathway is called the acrylate pathway (Fig. 2). This is applied for the conversion of lactate to propionate [15]. This reduction allows the cell to balance the anaerobic oxidation of lactate to acetate and carbon dioxide, which appears to be the primary source of ATP generation. Key steps of the pathway are catalyzed by the following enzymes sequentially: propionyl-CoA transferase (EC 2.8.3.1) lactyl-CoA dehydratase (EC 4.2.1.54) and acrylyl-CoA reductase (EC 1.3.1.95). Propionyl-CoA transferase typically catalyzes the interconversion of DL-lactate/DL lactyl-CoA (Fig. 2 first step) and propionate/propionyl-CoA translocations (Fig. 2 fourth step). This CoA-transferase catalyzes the transfer of the CoA moiety from propionyl-CoA to lactate. Lactyl-CoA dehydratase converts lactyl-CoA to acrylyl-CoA (Fig. 2 second step). Propionyl-CoA is generated from Acrylyl-CoA by acrylyl-CoA reductase (Fig. 2 third step) [9, 16]. The acrylate pathway for propionate production is applied by *Clostridium propionicum* [16, 17], *Coprococcus catus* [15], *Clostridium homopropionicum* [13], *Megasphaera elsdenii* [16] and *Prevotella ruminicola* [17-19].

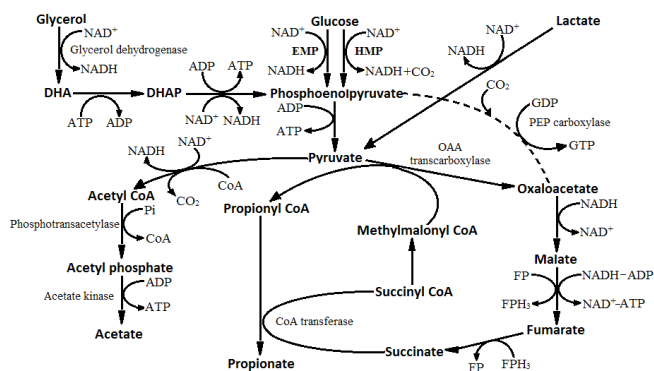


Fig. 1 Succinate pathway in *Propionibacterium*. The biosynthetic pathway from glycerol to propionic acid proceeds from glycerol to dihydroxyacetone (DHA) to dihydroxyacetone phosphate (DHAP) to phosphoenolpyruvate (PEP) to pyruvate and oxaloacetate and from oxaloacetate to malate, fumarate, succinate, succinyl coenzyme A (CoA), methylmalonyl CoA, propionyl CoA, and propionate (see arrows). Acetate is the main byproduct in the production of propionic acid. The figure was copied from [14] with permission.

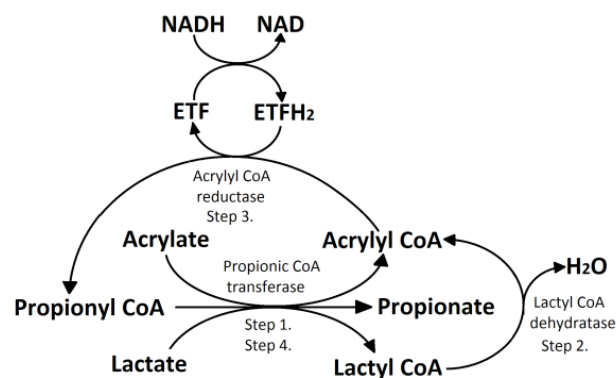


Fig. 2 Acrylate pathway for the metabolism of lactate into propionate by *Megasphaera elsdenii*. This pathway is modified to show acrylate consumption via propionyl-CoA transferase. ETF: Electron transfer flavoprotein. Figure was taken from [16] with permission.

2.3 Propanediol pathway

Several different bacteria are known to produce 1,2-propanediol from fucose, rhamnose or lactate. In some bacteria 1,2-propanediol can be further metabolized to propionate (Fig. 3) or propanol, e.g. *Salmonella enteric serovar Typhimurium* and *Roseburia inulinivorans* [6]. Fucose is converted to 1,2-propanediol through L-fucose, L-fuculose, Fuculose 1-phosphate and L-lactaldehyde. The enzymes are L-fucose isomerase (EC 5.3.1.25), L-fuculokinase (EC 2.7.1.51), L-fuculose phosphate aldolase (EC 4.1.2.17) and propanediol oxidoreductase (EC 1.1.1.77). Then the next step is the conversion of 1,2-propanediol to propionaldehyde by propanediol dehydratase (EC 4.2.1.28). Afterwards, propionaldehyde is catabolized to propionyl-CoA and propanol by propionaldehyde dehydrogenase (EC 1.2.1.87) and propanol dehydrogenase (EC 1.1.1.1), respectively. The propionyl-CoA is converted to propionyl

phosphate and then to propionate by phosphotransacetylase (EC 2.3.1.222) and propionate kinase (EC 2.7.2.15) [20]. This pathway provides a source of ATP and carbon compounds that can be diverted to central metabolism via known pathways. The propanediol pathway is also present in *Ruminococcus obeum* [7] and *Bacteroides thetaiotaomicron* [15-20].

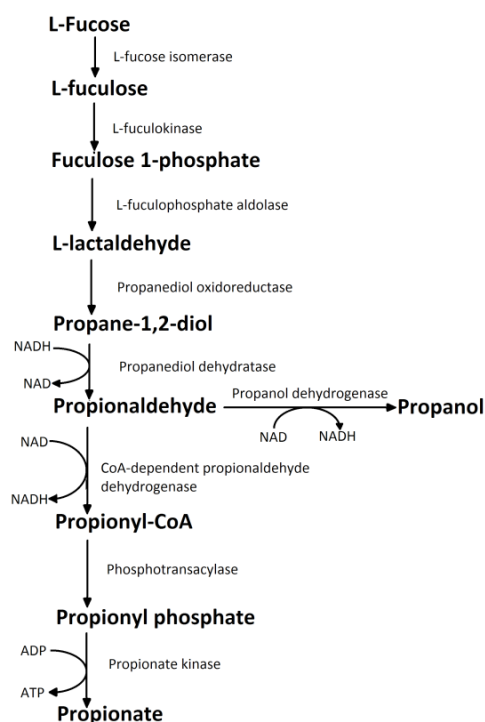


Fig. 3 Propanediol pathway for L-fucose conversion to propionate via 1,2-propanediol. Figure was taken from [20] with permission.

2.4 Comparison of ATP production and redox-balance during the different propionate pathways

The straightforward propionic acid pathway should be redox-balanced itself and be able to generate ATP to avoid the need of byproduct formation, otherwise the microbes pursue the redox-balance via by-product formation decreasing the yield of propionic acid. As shown in Table 1 none of the pathways can fulfil these criteria resulting from heterofermentative behaviour.

In the succinate pathway one mole of ATP and one mole of NADH per mole of propionate are produced but one mole of ATP is utilized and two moles of NADH are consumed if the substrate is glucose. In the case of lactate no ATP production occurs, the net ATP production is -1. If the substrate is glycerol the production and utilization of ATP and NADH is balanced.

During the acrylate pathway the net production of ATP is 0 and one mole of NADH is generated while two moles are consumed if the substrate is glucose. In lactate utilization one mole of ATP and one mole of NADH is required per mole of propionate. In the case of glycerol the net production of ATP is 0 and the process is redox-balanced.

The propanediol pathway produces and utilizes one mole of ATP and generates one mole but consumes two moles of NADH per mole of propionate from L-fucose. There is no literature about propionate production from lactose, glucose or glycerol through the propanediol pathway. In this novel pathway if the substrate conversion to 1,2-propanediol is connected with the 1,2-propanediol conversion to propionate, in all cases the consumption of NADH is greater than its production. The net ATP is 0 when the substrate is glucose or glycerol and 1 when lactate is utilized.

Table 1 Net ATP and redox-balance of propionate-producing pathways. +1 – configuration would be favourable.

Pathway	Substrate	Net ATP*	Redox-balance
Succinate	Glucose	0	-1
	Lactose	-1	-1
	Glycerol	0	0
Acrylate	Glucose	0	-1
	Lactose	-1	-1
	Glycerol	0	0
Propanediol	Glucose	0	-1
	Lactose	1	-1
	Glycerol	0	-1
	Fucose/Rhamnose	0	-1

*Net ATP yield mol/mol propionate

3 Biotechnological production

Beside the fact that fermentative production provides the opportunity for countries with limited petroleum feedstocks to manufacture propionic acid, it also provides a more sustainable approach [1]. Currently, propionic acid processes that are bio-based are limited because of their low levels of productivity, yield and final propionic acid concentration which are caused by strong end-product inhibition. Nowadays, production of propionic acid via petrochemical routes is more economical than applying biotechnological methods. For economical biotechnological production, it is necessary to increase the bacterial tolerance to propionic acid. There are reported solutions that increase this tolerance. One of them is serial adaptation followed by screening and isolation of single colonies on propionate-containing agar plates [21]. Genetic engineering provides new tools for generating mutants with improved fermentation capabilities [3, 22], e.g. metabolically engineered mutants (ACK-Tet) were generated to tolerate and produce propionic acid thus the yield of propionic acid increased by 13% in comparison to wild type [23]. Derivates of sucrose, glucose, glycerol or cellulose as abundant and economical feedstocks are required for propionic acid fermentation processes.

Cellulosics-based feedstocks are cheap, are exposed to reduced levels of competition with food sources and are widely available. Their only downside is the limited number of solutions for industrial scale applications. Glucose- and sucrose-based propionic acid fermentations have been widely studied. Some research achieved over 100 g*L⁻¹ propionic acid titres [1]. Several bacteria like *Propionibacteria*, *Clostridium propionicum* and many others can produce propionic acid under anaerobic conditions. Among the reported cases, higher yields are achieved with the genus of *Propionibacteria* (Table 2).

Propionibacteria are Gram-positive, nonmotile, non-spore-forming facultative anaerobes. They usually form wet, bright and oily, round or granular colonies of a creamy, red, brown or orange colour. The genus of *Propionibacteria* is divided into two groups: dairy and cutaneous *Propionibacteria* [2]. Dairy *Propionibacteria* are widely used in the production of Swiss cheese, vitamin B12 and propionic acid. They are non-toxic and non-pathogenic; products from these bacteria are safe for human consumption [9, 24]. *Propionibacteria* can be utilized for the growth and product formation of several carbon sources, e.g. glucose, lactose, lactate, xylose, arabinose, glycerol and sorbitol. As a nitrogen source organic sources are mainly used such as yeast extract, trypticase soy broth, corn steep liquor, soybean meal and tryptone. *Propionibacteria* usually require biotin and pantothenic acid as vitamin supplements in their growth media [25]. The optimal temperature for cell growth ranges from 30 to 32°C. *Propionibacteria* grow most rapidly at pH's of between 6.0 and 7.0, while no growth was observed at pH's of below 4.5 [26].

To improve the yield and productivity of propionic acid in *Propionibacteria*, extensive studies have been conducted. Various carbon and nitrogen sources have been examined and several cultivation techniques tested [8]. Since both the availability of genomic information and tools of synthetic biology with regard to *Propionibacteria* have improved recently, it has become a reality to increase the production of propionic acid with metabolically engineered *Propionibacteria* [8]. There have been many metabolic engineering studies reported in recent years [25, 27, 28]. Propionic acid titres have been improved by overexpressing enzymes such as glycerol dehydrogenase, malate dehydrogenase [2] and fumarate hydratase in *Propionibacterium jensenii*, phosphoenolpyruvate carboxylase (PPC) in *Propionibacterium freudenreichii*, and propionyl-CoA:succinate CoA transferase in *Propionibacterium shermanii* [8]. The yield of propionic acid was also increased by the inactivation of the acetate kinase gene [23]. In a study, gene-encoding phosphoenolpyruvate carboxylase (PPC) was cloned from *Escherichia coli* and expressed in *Propionibacterium freudenreichii*. PPC catalyzes the conversion of phosphoenolpyruvate to oxaloacetate with the fixation

of one molecule of CO₂. Its expression in *P. freudenreichii* exhibited profound effects on propionic acid fermentation. Compared to the wild type, the mutant expressing the *ppc* gene grew significantly faster, consumed more glycerol, and produced propionate of a larger final titre at a faster rate, i.e. productivity increased by 46% [29].

To eliminate such end-product inhibition, various extractive fermentation processes have been developed to continuously remove propionic acid from the fermentation medium resulting in the product yield increasing by 24% [30].

There are also metabolic engineering solutions to protect *Propionibacteria* against propionic acid stress. Two key acid resistance elements, the arginine deaminase and glutamate decarboxylase systems, have been identified and modified to improve acid resistance by 10 fold and even propionic acid production by 20% with regard to *Propionibacterium jensenii* [31].

3.1 Propionic acid yield

In Table 2 the highest propionic acid yield is 0.90 g*g⁻¹ [35]. This was achieved with *Clostridium neopropionicum* which was isolated from a mesophilic anaerobic digester treating industrial vegetable cannery wastewater. Fermentation was conducted in 25 ml vials containing 10 ml of medium under a N₂/CO₂ (80% : 20%) headspace. The initial concentration of ethanol was 30 mM (1.38 g*L⁻¹) and the produced propionic acid concentration was 1.25 g*L⁻¹. Also the second and third highest values were achieved in this report [35].

Propionibacterium freudenreichii could also achieve a high propionic acid yield of 0.71 g*g⁻¹ [46]. In this report batch fermentations were studied in three parallel 200 mL expanded bed adsorption bioreactors (EBAB) connected with a recirculation loop to a 5 L stirred-tank fermenter to control the temperature and pH. The EBAB was made of a glass column packed with Duolite A30 resin, and both ends of the column were equipped with a stainless steel wire mesh. In this process, propionic acid was adsorbed semicontinuously from the broth, and replenishment with fresh resin was possible when needed by alternating the three columns. The fermentation broth with free cells was circulated through the EBA column to separate the propionic acid in the expanded bed every 12–24 h, when 10 g*L⁻¹ propionic acid was produced. To optimize the fermentation performance, a strategy of gradually adding feedstock was employed, in which glycerol was introduced at set times (12, 24, 48, and 72 h) with a constant mass ratio of 2.5 to glucose. When glycerol was added after 48 h, the highest product concentration (propionic acid: 42.7 g*L⁻¹), the highest yield (propionic acid: 0.71 g*g⁻¹), and highest productivity (propionic acid: 0.36 g*L⁻¹*h⁻¹) of this experiment were obtained [46].

Table 2 Propionic acid producing microorganisms.

Strain	Fermentation technique/ volume/ substrate	PA titre (g*L ⁻¹)	PA productivity (g*L ⁻¹ *h ⁻¹)	Yield (g*g ⁻¹)	Ref.
<i>Anaeroglobus geminatus</i> AIP313.00	Batch fermentation/n.a./ glucose	0.04	~0	0.01	[32]
<i>Bacteroides fragilis</i> DSMZ 2151	Batch fermentation/ 0.01L / glucose	0.37	0.005	0.07	[33]
<i>Bacteriodes thetaiotaomicron</i>	Batch fermentation/ 0.15L/ pectin	0.36	0.015	0.07	[34]
<i>Clostridium homopropionicum</i> DSM 5847	Batch fermentation/1L / lactate	1.06	n.a.	0.70	[13]
<i>Clostridium neopropionicum</i> DSM 3847	Batch fermentation/ 0.01L/ ethanol	1.25	0.02	0.90	[35]
	Batch fermentation/ 0.08 L/ ethanol	0.70	~0	0.76	[36]
	Batch fermentation/ 0.08 L/ propanol+acetate	1.34	0.01	0.75	[36]
<i>Eubacterium hallii</i> DSM 3353	Batch fermentation/ 0.01L/glucose	1.48	0.01	0.12	[37]
<i>Megasphaera elsdenii</i> ATCC 17753	Batch fermentation/ 0.1L / DL-lactate	0.35	0.02	0.01	[16]
<i>Megasphaera elsdenii</i> NIAH 1102	Batch fermentation/ 1L/ DL-lactate	0.62	0.05	0.17	[38]
<i>Negativicoccus succinicivorans</i>	Batch fermentation/n.a./ glucose + sodium succinate	3.49	0.05	n.a.	[12]
<i>Pelobacter propionicus</i> DSM 2379	Batch fermentation/ 1L / lactate	0.26	n.a.	0.52	[13]
<i>Prevotella ruminicola</i> 23	Batch fermentation/ 1L/ glucose	0.59	n.a.	0.30	[18]
<i>Propionibacterium acidipropionici</i> ACT-1	Sequential batch fermentation with cell recycle/n.a./ glucose	39.7	2.98	0.46	[21]
	Fed batch fermentation with cell recycle/n.a. / glucose	55.7	2.23	0.43	[21]
<i>Propionibacterium acidipropionici</i> ATCC 4875	Fed-batch fermentation with cell recycle/ 0.1L/ glucose	70.8	0.42	0.36	[39]
	Fed-batch/ 3L/ corncob molasses	71.8	0.28	n.a.	[40]
	Batch fermentation/ 2L/ glycerol	23.55	0.12	0.70	[41]
	Fibrous-bed bioreactor, fed batch fermentation/ 2L/ glucose	71.8	0.08	0.4	[42]
	Fed-batch extractive fermentation/ 2L/ lactose	75	0.98	0.66	[43]
	Fibrous-bed bioreactor, fed batch fermentation/ 2L/ glycerol	106	0.04	~0.50	[3]
<i>Propionibacterium acidipropionici</i> CGMCC1.2230	Fed-batch fermentation/ 3L/ glycerol	23.1	0.10	0.46	[4]
<i>Propionibacterium acidipropionici</i> DSM 4900	Batch fermentation/ 1L/ glycerol	43.40	0.35	0.62	[44]
<i>Propionibacterium acidipropionici</i> NRRL B-3569	Continuous fermentation with packed bed column/ 1L/ glycerol	7.7	0.88	0.58	[24]
<i>Propionibacterium acidipropionici</i> WSH1105	Fed-batch fermentation/ 3L/ glycerol	27.1	0.13	0.45	[4]
<i>Propionibacterium freudenreichii</i> CCTCC M207015	Plant fibrous-bed bioreactor, fed-batch fermentation / 7.5L/ sugarcane molasses	91.89	0.36	0.46	[45]
<i>Propionibacterium freudenreichii</i> CICC 10019	Batch fermentations in expanded bed adsorption bioreactor/ 0.2L/ glucose +glycerol	42.7	0.36	0.71	[46]
<i>Propionibacterium freudenreichii</i> DSM 20271 mutant Pf(ppc)	Batch fermentation/ 0.5L/ glucose	19.5	0.10	0.51	[29]
<i>Propionibacterium jensenii</i> (pZGX04-ppc)	Fed-batch fermentation/ 3L/ residual glycerol	33.21	0.15	0.66	[8]
<i>Propionibacterium jensenii</i> (pZGX04-gldA-mdh)	Fed-batch fermentation / 2L/ glycerol	36.09	0.17	0.65	[2]
<i>Roseburia inulinivorans</i> DSM 16841	Batch fermentation/ n.a./ fucose	1.39	0.09	0.28	[20]
<i>Selenomonas ruminantium</i> HD4	Batch fermentation/ 0.037L/ DL-lactate	1.3	0.01	0.13	[47]
<i>Veillonella parvula</i> DSM 2008	Batch fermentation/ 1L/ Lactate	1.07	n.a.	0.59	[13]

3.2 Propionic acid titre

As shown in Table 2 larger values of propionate concentration are achieved by *Propionibacterium* species. The highest propionic acid titre is $106 \text{ g}\cdot\text{L}^{-1}$ which was reported by An Zhang and Shang-Tian Yang [3]. In this experiment researchers used a mutant strain of *Propionibacterium acidipropionici* ATCC 4875 with acetate kinase (*ack*) gene knock-out. The medium contained $40 \text{ g}\cdot\text{L}^{-1}$ of glycerol as a carbon source. The immobilized-cell fermentation was studied in a fibrous-bed bioreactor (FBB) which was connected to a 5 L fermenter to control the pH and temperature through a recirculation loop. A piece of cotton cloth was spirally wound with a stainless steel mesh and packed into the glass column. The FBB itself had a working volume of $\sim 600 \text{ mL}$ and the complete system contained $\sim 2 \text{ L}$ of the medium. After inoculation, the FBB was operated under the repeated batch mode for several batches with glucose as the substrate to increase the cell density in the reactor system. After the cell density had reached the desired value of glucose, the glycerol feed was started. The fermentation lasted for 4 months which equates to 3000 hours. The glycerol fed-batch fermentation was conducted five times. The long fermentation time resulted in low propionic acid productivity ($0.04 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) contrary to the highest propionic acid titre ($106 \text{ g}\cdot\text{L}^{-1}$) [3].

The second highest propionic acid titre in Table 2 was $91.89 \text{ g}\cdot\text{L}^{-1}$ [45]. In this study *Propionibacterium freudenreichii* CCTCC M207015 was used. The fermentation was conducted in a plant fibrous-bed bioreactor (PFB) which was composed primarily of a 7.5 L stirred-tank fermenter (BioFlo 110, New Brunswick Scientific, USA) and immobilized cells in a glass column (ID: 4 cm; and height 60 cm). The column and stirred-tank fermenter were connected with a peristaltic pump. The chopped bagasse was dried to achieve constant weight and loaded into the glass column with an estimated weight of approximately 30 g for immobilization of the cells. The bagasse was kept in place using a stainless steel wire mesh on top and at the bottom of the column to avoid leakage. The fermentation used hydrolysed cane molasses as the medium. Cane molasses contained 35.8% (w/w) sucrose, 5.2% (w/w) glucose, 8.7% (w/w) fructose, 2.5% (w/w) other carbohydrates, 4.3% (w/w) crude protein, 0.1% (w/w) crude fat, 9.6% (w/w) ash, 4.6% (w/w) salt, 8.9% (w/w) metal ions such as calcium, potassium, sodium, iron, magnesium and copper, and 20.3% (w/w) water. During the fed-batch fermentation when the sugar concentrations of the broth decreased to $10 \text{ g}\cdot\text{L}^{-1}$, a concentrated sugar solution of $500 \text{ g}\cdot\text{L}^{-1}$ was fed into the PFB to maintain the sugar concentration at around $10 \text{ g}\cdot\text{L}^{-1}$ using a constant flow pump. The highest value for final propionic acid production was $91.89 \text{ g}\cdot\text{L}^{-1}$, which was achieved over 254 hours. The propionic acid yield was $0.46 \text{ g}\cdot\text{g}^{-1}$ and productivity $0.36 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ [45].

3.3 Propionic acid productivity

The highest propionic acid productivity was achieved by sequential batch (hereinafter SB) fermentation with cell recycling [21]. *Propionibacterium acidipropionici* ACT-1 was used in the study. Cells in the late exponential phase (cultured in 3 L of medium with $50 \text{ g}\cdot\text{L}^{-1}$ glucose at pH 6.5) were collected by centrifugation and used to seed the batch fermentation in a 1 L bioreactor containing 500 mL of medium agitated at 100 rpm. A total of four sequential batches were performed at pH 6.5 for a total duration of 65 h. In the SB fermentation with high initial cell density, the propionic acid yield was stable at $0.45 \text{ g}\cdot\text{g}^{-1}$ and the volumetric productivity increased from $2.06 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ for the first batch to $2.74 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ for the fourth batch, which was also proportional to the average cell density in these SB fermentations. It is noted that a significantly higher yield of $0.48 \text{ g}\cdot\text{g}^{-1}$ and productivity of $\sim 3 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ were obtained in the third batch, which achieved the highest cell density of $\sim 49 \text{ g}\cdot\text{L}^{-1}$ in terms of the dry weight of the cell (corresponding to OD 244.8). The highest propionic acid titre of $42.7 \text{ g}\cdot\text{L}^{-1}$ obtained in the second batch also confirmed that ACT-1 had a relatively high tolerance to propionate at pH 6.5 [21].

The second highest propionic acid productivity in Table 2 is $2.23 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ achieved by fed-batch fermentation also with cell recycling [21]. *Propionibacterium acidipropionici* ACT-1 was used in the study, as well. Fermentation was performed in a 1 L bioreactor containing 500 mL of medium agitated at 100 rpm. The temperature of the bioreactor was maintained at 32°C and the pH was controlled at 6.5 by the addition of ammonium hydroxide. An initial glucose concentration of $\sim 50 \text{ g}\cdot\text{L}^{-1}$ was used and a concentrated glucose solution was pulse fed into the reactor when the glucose concentration was less than $\sim 15 \text{ g}\cdot\text{L}^{-1}$ or had almost depleted. The final propionic acid titre at pH 6.5 achieved $55.7 \text{ g}\cdot\text{L}^{-1}$ with a high productivity of $2.23 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ and a propionic acid yield of $0.43 \text{ g}\cdot\text{g}^{-1}$. Both the productivity and yield were comparable to those of high cell density sequential batch fermentations at pH 6.5, suggesting that ACT-1 can tolerate a propionic acid concentration in excess of $50 \text{ g}\cdot\text{L}^{-1}$ at pH 6.5 [21].

4 Propionic acid recovery

It is really hard to make a compose a comprehensive review on downstream operations because their effectivity is strongly determined by the different feedstocks (yielding the matrix), the producer strain (that determines accompanying by-products), or the required final product quality and formula. Additionally, existing recovery reports focus either on recovery after synthetic production or recovery from model solutions, but almost none of them address recovery from real fermentation broths. However we compared the available processes on the basis of their key operations, and the overall recovery yields obtained.

The importance of propionic acid production by fermentation makes it necessary to develop new, efficient methods for

propionic acid separation from fermentation broths or requires substantial improvement in existing recovery technologies. Around half of the total production costs are associated with downstream processing [48], therefore these improvements have a similar impact to developments on fermentation processes. As a result this overview introduces the possible processes with regard to model solutions. Since the purity of the product is often required to be in excess of 99.5% for propionic acid, a high extent of recovery is needed in the downstream process. Low energy consumption as well as low consumption of chemicals is necessary during product recovery. Therefore efficient levels of mass and heat transfer must be exhibited by the recovery equipment. To meet all of these requirements the downstream process has to fulfil the following key requirements:

- In clarification: removal of large particles.
- In primary recovery: removal of the product from the bulk aqueous solution containing major impurities.
- Counterion removal: replacing the cation of a propionate with H^+ to produce propionic acid (if required).
- Concentration/purification: removal of the bulk solvent or capture of the propionic acid, achieving the concentration as well as removal of remaining impurities [49].

4.1 Clarification

Cell removal is usually the first step of the downstream process step and is achieved by filtration or centrifugation, as in the case for other fermentation products [9].

4.2 Primary recovery

The aqueous propionic acid or propionate solution generally contains several impurities such as sugars, salts, fermentation by-products and debris derived from the cell lysis. A good recovery strategy is the transfer of the product to another phase, namely the extractant phase, adsorbent phase, precipitate phase, or an aqueous phase behind a membrane. Several separation methods are known and have been applied for primary recovery such as liquid-liquid extraction, ultra filtration, reverse osmosis, electro-dialysis, direct distillation, liquid surfactant membrane extraction, anion exchange, precipitation, and adsorption [50].

In the literature concerning the primary recovery of carboxylic acids, adsorption and extraction dominate. Numerous options have been suggested, due to the wide variety of interactions between carboxylic acids and amine-based adsorbents and extractants [51]. It is necessary to regenerate the adsorbents and extractants because of their expense compared to the recovered carboxylic acids, and the high costs of treating them as waste. Adsorbents have very different features to extractants such as the equipment used, costs, process losses and safety aspects. While adsorption is usually conducted by packed beds,

extraction generally utilizes different types of stirred reactors [52, 53]. For extraction, contact between the phases is necessary, which may also be achieved via membranes, resulting in membrane-based solvent extraction, supported liquid membranes and pertraction as the most typical modes [54, 55].

Reactive extraction is also one of the applicable separation processes. It is based on the reactions between extractants and the materials extracted. The extractant in the organic phase reacts with the material in the aqueous phase and the product formed is then dissolved in the organic phase, too. Extractants such as hydrocarbons, phosphorous, and aliphatic amines are mainly used in the reactive extraction of carboxylic acids [56]. Investigations have been conducted into the reactive extraction of propionic acid using tri-n-octylamine in sCO_2 [48].

During precipitation a soluble carboxylic acid (propionic acid) or soluble carboxylate (propionate) is converted into an insoluble carboxylate by a double replacement reaction. The conventional industrial method of carboxylic acid separation is precipitation using calcium hydroxide. This method is environmentally unfriendly and expensive. It requires large amounts of sulphuric acid and generates a solid by-product in the form of calcium sulphate [48, 51].

Nanofiltration and reverse osmosis are pressure-driven membrane techniques. The passage of water and somewhat larger molecules is allowed by nanofiltration membranes. Ions rather than neutral molecules of the same size are retained, which indicates a potential for concentrating as well as purifying carboxylic acid or carboxylate solutions. Reverse osmosis membranes have smaller pores which mainly allow the permeation of water, resulting in a concentration step, as well. Microfiltration and ultrafiltration membranes may not prevent the passage of propionic acid effectively and sufficiently because of its low molecular weight [57].

During conventional electrodialysis a feed solution of a carboxylate salt is introduced between cation and anion exchange membranes. Driven by an electrostatic potential, cations and anions diffuse in opposite directions, but they are only able to pass through a cation or anion exchange membrane, respectively. This results in a more concentrated solution and a more dilute solution of carboxylate salt [51, 58].

4.3 Removal of counterion

In many cases carboxylate salts are formed whereas carboxylic acids are desired. When converting carboxylate into carboxylic acid, H^+ has to replace the counterion of the carboxylate (e.g. Na^+). Depending on the required procedure, the removal will lead to a (sodium) salt or base as a co-product. This co-product can be removed from the carboxylic acid if both products end up in different phases. The counterion can be removed by precipitation, ion exchange adsorbents, or extractants [49].

Table 3 Samples for propionic acid recovery from model aqueous solution using primary recovery methods

Method	Used adsorbent, extractant and solvent	removal of acid %	Ref.
Adsorption	Amberlite IRA-67	84.46	[53]
Adsorption	alumina	16.94	[59]
Electrodialysis	Acid Dialyzer GII00	82.65	[60]
Extraction	Aliquat 336 in Cyclohexane	63.25	[61]
Extraction	Aliquat 336 in Hexane	61.53	[61]
Extraction	Aliquat 336 in Ethyl acetate	72.65	[61]
Extraction	Aliquat 336 in Toluene	66.67	[61]
Extraction	Aliquat 336 in Methyl isobutyl ketone	70.94	[61]
Reactive Extraction	Aliquat 336 in 1-decanol	66.68	[62]
Reactive Extraction	tri-n-octylamine + oleyl alcohol	91.00	[63]
Reactive Extraction	tri-n-butyl phosphate + kerosene	81.25	[64]
Extraction	Hexanol	80.99	[54]
Extraction	2-Octanol	80.33	[54]

4.4 Water removal and carboxylic acid purification

Water is the major accompanying by-product, therefore its removal is determinative for recovery costs. A large proportion of the water may be removed during primary recovery, but often a separate water removal step has to be introduced of which several methods are available. These may be used at different points in the process, to concentrate the carboxylic acid, its salt, or an inorganic salt. The removal of water is often integrated with purification by distillation or crystallization [52].

Since reverse osmosis (RO) membranes only allow the passage of water, a concentration effect is obtained that may be exploited for crystallization. This RO may be energetically more favourable than heat-driven evaporation but has other limitations. The proportion of permeated carboxylic acid should be negligible, otherwise a loss of product would result [57].

During evaporation, water and possibly volatile impurities are removed from non-volatile carboxylic acid. Evaporation is the default process for removing water. Evaporation costs are high for dilute aqueous solutions due to the energy required to evaporate water. Therefore it is important to obtain concentrated solutions by fermentation and to concentrate rather than dilute during primary recovery and counterion removal [49].

4.5 In-situ product removal

In-situ removal of the acids from the fermentation broth could be one of the solutions to reduce product inhibition. Removing carboxylic acid or carboxylate during fermentation can prolong the production time before the fermentation stops due to product inhibition, resulting in improved productivity. Moreover, removing the undissociated carboxylic acid can reduce the consumed amount of pH-controlling base and the associated inorganic salt production [65, 66].

5 Conclusions and future developments

Fermentation using renewable resources is an attractive alternative route of propionic acid production, although commercial production is mostly conducted by chemical synthesis from petroleum feedstocks. Currently propionic acid fermentation cannot compete with petrochemical routes due to its relatively low productivity, yield, final titre, and product purity. Many attempts have been made to overcome these problems on both organism and process levels. The genomes of several *Propionibacteria* species have been fully sequenced, and the synthetic pathways of propionic acid elucidated. Overexpression and knockout vectors have been developed as useful tools for the metabolic engineering of *Propionibacteria*. With the recent advances in “omic” technologies and systems biology, new tools and abundant genetic and metabolic data can be generated expediently, including genome-scale metabolic modelling and metabolic flux analysis. Furthermore these advances have also been used to explore metabolically important enzymes. Biosynthetic pathways of propionic acid can be re-engineered with precisely targeted and manipulated enzymes to greatly improve fermentation. On the process level, several cheap and readily available carbon as well as nitrogen sources have been identified to support good degrees of cell growth and propionic acid production. Most of them are agricultural residues and processing wastes. In terms of process engineering, cell recycling and immobilization are promising techniques to achieve high cell densities, high product titres, high productivity, and high yields simultaneously, while at the same time the process can be operated stably for an extended period. As shown in Table 2 the major values are achieved by methods that utilize cell recycling and high cell densities. Finally, fermentation integrated with separation for online or in-situ product recovery will serve as a driving force to further improve product titres, yields, and productivity [9, 67].

References

- [1] Stowers, C. C., Cox, B. M., Rodriguez, B. A. "Development of an industrializable fermentation process for propionic acid production." *Journal of Industrial Microbiology and Biotechnology*. 41(5), pp. 837–852. 2014. <https://doi.org/10.1007/s10295-014-1423-6>
- [2] Liu, L., Zhuge, X., Shin, H. D., Chen, R. R., Li, J., Du, G., Chen, J. "Improved production of propionic acid in *Propionibacterium jensenii* via combinational overexpression of glycerol dehydrogenase and malate dehydrogenase from *Klebsiella pneumoniae*." *Applied and Environmental Microbiology*. 81(7), pp. 2256–2264. 2015. <https://doi.org/10.1128/AEM.03572-14>
- [3] Zhang, A., Yang, S. T. "Propionic acid production from glycerol by metabolically engineered *Propionibacterium acidipropionici*." *Process Biochemistry*. 44(12), pp. 1346–1351. 2009. <https://doi.org/10.1016/j.procbio.2009.07.013>
- [4] Guan, N., Li, J., Shin, H. D., Wu, J., Du, G., Shi, Z., Liu, L., Chen, J. "Comparative metabolomics analysis of the key metabolic nodes in propionic acid synthesis in *Propionibacterium acidipropionici*." *Metabolomics*. 11(5), pp. 1106–1116. 2014. <https://doi.org/10.1007/s11306-014-0766-3>
- [5] MarketResearchStore.Com. "Global Propionic Acid Market Set for Explosive Growth, To Reach Around USD 1.55 Billion by 2020, Growing at 7% CAGR." 2016. [Online]. Available from: <https://globenewswire.com/news-release/2016/02/16/810981/0/en/Global-Propionic-Acid-Market-Set-for-Explosive-Growth-To-Reach-Around-USD-1-55-Billion-by-2020-Growing-at-7-CAGR-MarketResearchStore-Com.html> [Accessed: 20th February 2017]
- [6] Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K. P., Flint, H. J., Louis, P. "Phylogenetic distribution of three pathways for propionate production within the human gut microbiota." *The ISME Journal*. 8(6), pp. 1323–1335. 2014. <https://doi.org/10.1038/ismej.2014.14>
- [7] Louis, P., Hold, G. L., Flint, H. J. "The gut microbiota, bacterial metabolites and colorectal cancer." *Nature Reviews Microbiology*. 12(10), pp. 661–672. 2014. <https://doi.org/10.1038/nrmicro3344>
- [8] Liu, L., Guan, N., Zhu, G., Li, J., Shin, H., Du, G. "Pathway engineering of *Propionibacterium jensenii* for improved production of propionic acid." *Nature Publishing Group*, 6(19963), pp. 1–9. 2016. <https://doi.org/10.1038/srep19963>
- [9] Wang, Z., Sun, J., Zhang, A., Yang, S.-T. "Propionic acid fermentation." In: Yang, S.-T., El-Enshasy, H. A., Nuttha, T. (eds.) *Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers*. (pp. 331–350), Wiley, 2013.
- [10] Parizzi, L. P., Grassi, M. C. B., Llerena, L. A., Carazzolle, M. F., Queiroz, V. L., Lunardi, I., Zeidler, A. F., Teixeira, P. J. P. L., Mieczkowski, P., Rincones, J., Pereira, G. A. G. "The genome sequence of *Propionibacterium acidipropionici* provides insights into its biotechnological and industrial potential." *BMC Genomics*. 13(562), pp. 1–20. 2012. <https://doi.org/10.1186/1471-2164-13-562>
- [11] Macy, J. M., Probst, I. "The biology of gastrointestinal bacteroides." *Annual Review of Microbiology*. 33(4), pp. 561–594. 1979. <https://doi.org/10.1146/annurev.mi.33.100179.003021>
- [12] Marchandin, H., Teyssier, C., Campos, J., Jean-Pierre, H., Roger, F., Gay, B., Carlier, J.-P., Jumas-Bilak, E. "Negativicoccus succinicivorans gen. nov., sp. nov., isolated from human clinical samples, emended description of the family Veillonellaceae and description of Negativicutes classis nov., Selenomonadales ord. nov. and Acidaminococcaceae fam. nov. in the bacterial phylum Firmicutes." *International Journal of Systematic and Evolutionary Microbiology*. 60(6), pp. 1271–1279. 2010. <https://doi.org/10.1099/ijs.0.013102-0>
- [13] Seeliger, S., Janssen, P. H., Schink, B. "Energetics and kinetics of lactate fermentation to acetate and propionate via methylmalonyl-CoA or acrylyl-CoA." *FEMS Microbiology Letters*. 211(1), pp. 65–70. 2002. [https://doi.org/10.1016/S0378-1097\(02\)00651-1](https://doi.org/10.1016/S0378-1097(02)00651-1)
- [14] Zhuge, X., Liu, L., Shin, H., Chen, R. R., Li, J., Du, G., Chen, J. "Development of a propionibacterium-escherichia coli shuttle vector for metabolic engineering of propionibacterium jensenii, an efficient producer of propionic acid." *Applied and Environmental Microbiology*. 79(15), pp. 4595–4602. 2013. <https://doi.org/10.1128/AEM.00737-13>
- [15] Flint, H. J., Duncan, S. H., Scott, K. P., Louis, P. "Links between diet, gut microbiota composition and gut metabolism." *The Proceedings of the Nutrition Society*. 74(1), pp. 13–22. 2015. <https://doi.org/10.1017/S0029665114001463>
- [16] Prabhu, R., Altman, E., Eitemana, M. A. "Lactate and acrylate metabolism by *Megasphaera elsdenii* under batch and steady-state conditions." *Applied and Environmental Microbiology*. 78(24), pp. 8564–8570. 2012. <https://doi.org/10.1128/AEM.02443-12>
- [17] Kandasamy, V., Vaidyanathan, H., Djurdjevic, I., Jayamani, E., Ramachandran, K. B., Buckel, W., Jayaraman, G., Ramalingam, S. "Engineering *Escherichia coli* with acrylate pathway genes for propionic acid synthesis and its impact on mixed-acid fermentation." *Applied Microbiology and Biotechnology*. 97(3), pp. 1191–1200. 2013. <https://doi.org/10.1007/s00253-012-4274-y>
- [18] Strobel, H. J. "Vitamin B12-dependent propionate production by the ruminal bacterium *Prevotella ruminicola* 23." *Applied and Environmental Microbiology*. 58(7), pp. 2331–2333. 1992. <https://doi.org/0099-2240/92/072331>
- [19] Hosseini, E., Grootaert, C., Verstraete, W., Van de Wiele, T. "Propionate as a health-promoting microbial metabolite in the human gut." *Nutrition Reviews*. 69(5), pp. 245–258. 2011. <https://doi.org/10.1111/j.1753-4887.2011.00388.x>
- [20] Scott, K. P., Martin, J. C., Campbell, G., Mayer, C. D., Flint, H. J. "Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium "*Roseburia inulinivorans*"." *Journal of Bacteriology*. 188(12), pp. 4340–4349. 2006. <https://doi.org/10.1128/JB.00137-06>
- [21] Wang, Z., Jin, Y., Yang, S. T. "High cell density propionic acid fermentation with an acid tolerant strain of *Propionibacterium acidipropionici*." *Biotechnology and Bioengineering*. 112(3), pp. 502–511. 2015. <https://doi.org/10.1002/bit.25466>
- [22] Kiatpapan, P., Murooka, Y. "Genetic manipulation system in propionibacteria." *Journal of Bioscience and Bioengineering*. 93(1), pp. 1–8. 2002. [https://doi.org/10.1016/S1389-1723\(02\)80045-7](https://doi.org/10.1016/S1389-1723(02)80045-7)
- [23] Suwannakham, S., Huang, Y., Yang, S.-T. "Construction and Characterization of *ack* Knock-Out Mutants of *Propionibacterium acidipropionici* for Enhanced Propionic Acid Fermentation." *Biotechnology and Bioengineering*. 94(2), pp. 383–393. 2006. <https://doi.org/10.1002/bit.20858>
- [24] Wallenius, J., Pahimanolis, N., Zoppe, J., Kilpeläinen, P., Master, E., Ilvesniemi, H., Seppälä, J., Erikäinen, T., Ojamo, H. "Continuous propionic acid production with *Propionibacterium acidipropionici* immobilized in a novel xylan hydrogel matrix." *Bioresource Technology*. 197, pp. 1–6. 2015. <https://doi.org/10.1016/j.biortech.2015.08.037>
- [25] Wang, Z., Lin, M., Wang, L., Ammar, E. M., Yang, S. T. "Metabolic engineering of *Propionibacterium freudenreichii* subsp. *shermanii* for enhanced propionic acid fermentation: Effects of overexpressing three biotin-dependent carboxylases." *Process Biochemistry*. 50(2), pp. 194–204. 2015. <https://doi.org/10.1016/j.procbio.2014.11.012>

- [26] Wang, Z. "Process development and metabolic engineering for enhanced PA by *P. freudenreichii*." PhD Thesis, The Ohio State University, 2013.
- [27] Wei, P., Lin, M., Wang, Z., Fu, H., Yang, H., Jiang, W., Yang, S. T. "Metabolic engineering of *Propionibacterium freudenreichii* subsp. *shermanii* for xylose fermentation." *Bioresource Technology*. 219, pp. 91–97. 2016.
<https://doi.org/10.1016/j.biortech.2016.07.056>
- [28] Tanaka, Y., Kasahara, K., Izawa, M., Ochi, K. "Applicability of ribosome engineering to vitamin B12 production by *Propionibacterium shermanii*." *Bioscience, Biotechnology, and Biochemistry*. 81(8), pp. 1636–1641. 2017.
<https://doi.org/10.1080/09168451.2017.1329619>
- [29] Ammar, E. M., Jin, Y., Wang, Z., Yang, S. T. "Metabolic engineering of *Propionibacterium freudenreichii*: Effect of expressing phosphoenolpyruvate carboxylase on propionic acid production." *Applied Microbiology and Biotechnology*. 98(18), pp. 7761–7772. 2014.
<https://doi.org/10.1007/s00253-014-5836-y>
- [30] Yang, S. T., Huang, H., Tay, A., Qin, W., Guzman, L. De, San Nicolas, E. C. "Extractive Fermentation for the Production of Carboxylic Acids." In: Yang, S.-T. (ed.) *Bioprocessing for Value-Added Products from Renewable Resources*. (pp. 421–446.), Elsevier, Amsterdam, 2007.
<https://doi.org/10.1016/B978-044452114-9/50017-7>
- [31] Guan, N., Li, J., Shin, H. dong, Du, G., Chen, J., Liu, L. "Metabolic engineering of acid resistance elements to improve acid resistance and propionic acid production of *Propionibacterium jensenii*." *Biotechnology and Bioengineering*. 113(6), pp. 1294–1304. 2016.
<https://doi.org/10.1002/bit.25902>
- [32] Jumas-Bilak, E., Carlier, J., Henry, C., Carrie, C. "Anaeroglobus geminatus gen. nov., sp. nov., a novel member of the family Veillonellaceae." *International Journal of Systematic and Evolutionary Microbiology*. 2002(52), pp. 983–986. 2002.
- [33] Rios-Covian, D., Cuesta, I., Alvarez-Buylla, J. R., Ruas-Madiedo, P., Gueimonde, M., de Los Reyes-Gavilán, C. G. "Bacteroides fragilis metabolises exopolysaccharides produced by bifidobacteria." *BMC Microbiology*. 16(1), pp. 150–157. 2016.
<https://doi.org/10.1186/s12866-016-0773-9>
- [34] Dongowski, G., Lorenz, A., Anger, H. "Degradation of Pectins with Different Degrees of Esterification by *Bacteroides thetaiotaomicron* Isolated from Human Gut Flora Degradation of Pectins with Different Degrees of Esterification by *Bacteroides thetaiotaomicron* Isolated from Human Gut Flora." *Applied and Environmental Microbiology*. 66(4), pp. 1321–1327. 2000.
<https://doi.org/10.1128/AEM.66.4.1321-1327>
- [35] Tholozan, J. L., Touzel, J. P., Samain, E., Grivet, J. P., Prensier, G., Albagnac, G. "Clostridium neopropionicum sp. nov., a strict anaerobic bacterium fermenting ethanol to propionate through acrylate pathway." *Archives of Microbiology*. 157(3), pp. 249–257. 1992.
<https://doi.org/10.1007/BF00245158>
- [36] Ato, M., Ishii, M., Igarashi, Y. "Enrichment of amino acid-oxidizing, acetate-reducing bacteria." *Journal of Bioscience and Bioengineering*. 118(2), pp. 160–165. 2014.
<https://doi.org/10.1016/j.jbiosc.2014.02.003>
- [37] Engels, C., Ruscheweyh, H. J., Beerenwinkel, N., Lacroix, C., Schwab, C. "The common gut microbe *Eubacterium hallii* also contributes to intestinal propionate formation." *Frontiers in Microbiology*. 7(713), pp. 1–12. 2016.
<https://doi.org/10.3389/fmicb.2016.00713>
- [38] Hino, T., Shimada, K., Maruyama, T. "Substrate preference in a strain of *Megasphaera elsdenii*, a ruminal bacterium, and its implications in propionate production and growth competition." *Applied and Environmental Microbiology*. 60(6), pp. 1827–1831. 1994.
- [39] Liu, Z., Ge, Y., Xu, J., Gao, C., Ma, C., Xu, P. "Efficient production of propionic acid through high density culture with recycling cells of *Propionibacterium acidipropionici*." *Bioresource Technology*. 216, pp. 856–861. 2016.
<https://doi.org/10.1016/j.biortech.2016.06.023>
- [40] Liu, Z., Ma, C., Gao, C., Xu, P. "Efficient utilization of hemicellulose hydrolysate for propionic acid production using *Propionibacterium acidipropionici*." *Bioresource Technology*. 114, pp. 711–714. 2012.
<https://doi.org/10.1016/j.biortech.2012.02.118>
- [41] Zhang, A., Sun, J., Wang, Z., Yang, S. T., Zhou, H. "Effects of carbon dioxide on cell growth and propionic acid production from glycerol and glucose by *Propionibacterium acidipropionici*." *Bioresource Technology*. 175, pp. 374–381. 2015.
<https://doi.org/10.1016/j.biortech.2014.10.046>
- [42] Suwannakham, S., Yang, S. T. "Enhanced propionic acid fermentation by *Propionibacterium acidipropionici* mutant obtained by adaptation in a fibrous-bed bioreactor." *Biotechnology and Bioengineering*. 91(3), pp. 325–337. 2005.
<https://doi.org/10.1002/bit.20473>
- [43] Jin, Z., Yang, S. T. "Extractive fermentation for enhanced propionic acid production from lactose by *Propionibacterium acidipropionici*." *Biotechnology Progress*. 14(3), pp. 457–465. 1998.
<https://doi.org/10.1021/bp980026i>
- [44] Dishisha, T., Ibrahim, M. H. A., Cavero, V. H., Alvarez, M. T., Hatti-Kaul, R. "Improved propionic acid production from glycerol: Combining cyclic batch- and sequential batch fermentations with optimal nutrient composition." *Bioresource Technology*. 176, pp. 80–87. 2015.
<https://doi.org/10.1016/j.biortech.2014.11.013>
- [45] Feng, X., Chen, F., Xu, H., Wu, B., Li, H., Li, S., Ouyang, P. "Green and economical production of propionic acid by *Propionibacterium freudenreichii* CCTCC M207015 in plant fibrous-bed bioreactor." *Bioresource Technology*. 102(10), pp. 6141–6146. 2011.
<https://doi.org/10.1016/j.biortech.2011.02.087>
- [46] Wang, P., Jiao, Y., Liu, S. "Novel fermentation process strengthening strategy for production of propionic acid and vitamin B12 by *Propionibacterium freudenreichii*." *Journal of Industrial Microbiology and Biotechnology*. 41(12), pp. 1811–1815. 2014.
<https://doi.org/10.1007/s10295-014-1513-5>
- [47] Paynter, M. J., Elsdén, S. R. "Mechanism of propionate formation by *Selenomonas ruminantium*, a rumen micro-organism." *Journal of General Microbiology*. 61(1), pp. 1–7. 1970.
<https://doi.org/10.1099/00221287-61-1-1>
- [48] Henczka, M., Djas, M. "Reactive extraction of acetic acid and propionic acid using supercritical carbon dioxide." *The Journal of Supercritical Fluids*. 110, pp. 154–160. 2016.
<https://doi.org/10.1016/j.supflu.2015.11.018>
- [49] López-Garzón, C. S., Straathof, A. J. J. "Recovery of carboxylic acids produced by fermentation." *Biotechnology Advances*. 32(5), pp. 873–904. 2014.
<https://doi.org/10.1016/j.biotechadv.2014.04.002>
- [50] Kumar, S., Babu, B. V. "Propionic Acid Production via Fermentation Route using Renewable Sources." (n.d.)
- [51] Tugtas, A. E. "Fermentative Organic Acid Production and Separation." *Fen Bilimleri Dergisi*. 23(2), pp. 70–78. 2011.
- [52] Rodriguez, B. A., Stowers, C. C., Pham, V., Cox, B. M. "The production of propionic acid, propanol and propylene via sugar fermentation: an industrial perspective on the progress, technical challenges and future outlook." *Green Chemistry*. 16(3), pp. 1066. 2014.
<https://doi.org/10.1039/c3gc42000k>

- [53] Uslu, H., Inci, I., Bayazit, Ş. S., Göksel, D. "Comparison of solid liquid equilibrium data for the adsorption of propionic acid and tartaric acid from aqueous solution onto amberlite IRA-67." *Industrial and Engineering Chemistry Research*. 48(16), pp. 7767–7772. 2009. <https://doi.org/10.1021/ie9005639>
- [54] Wasewar, K. L., Keshav, A., Seema "Physical extraction of propionic acid". *International Journal of Research and Reviews in Applied Sciences*. 3(3), pp. 290–302. 2010. [Online]. Available from: http://www.arpapress.com/Volumes/Vol3Issue3/IJRRAS_3_3_08.pdf [Accessed: 5th March 2017]
- [55] Keshav, A., Wasewar, K. L. "Back extraction of propionic acid from loaded organic phase." *Chemical Engineering Science*. 65(9), pp. 2751–2757. 2010. <https://doi.org/10.1016/j.ces.2010.01.010>
- [56] Hong, Y. K., Hong, W. H., Han, D. H. "Application of reactive extraction to recovery of carboxylic acids." *Biotechnology and Bioprocess Engineering*. 6, pp. 386–394. 2001. <https://doi.org/10.1007/BF02932319>
- [57] Choi, J.-H., Fukushi, K., Yamamoto, K. "A study on the removal of organic acids from wastewaters using nanofiltration membranes." *Separation and Purification Technology*. 59, pp. 17–25. 2008. <https://doi.org/10.1016/j.seppur.2007.05.021>
- [58] Huang, C., Xu, T., Zhang, Y., Xue, Y., Chen, G. "Application of electrodialysis to the production of organic acids: State-of-the-art and recent developments." *Journal of Membrane Science*. 288(1–2), pp. 1–12. 2007. <https://doi.org/10.1016/j.memsci.2006.11.026>
- [59] Uslu, H. "Investigation of Adsorption Equilibrium and Kinetics of Propionic Acid and Glyoxylic Acid from Aqueous Solution by Alumina." *Chemical & Engineering Data*. 56, pp. 3301–3308. 2011. <https://doi.org/10.1021/je2000765>
- [60] Zhang, S. T., Matsuoka, H., Toda, K. "Production and recovery of propionic and acetic acids in electrodialysis culture of *Propionibacterium shermanii*." *Journal of Fermentation and Bioengineering*. 75(4), pp. 276–282. 1993. [https://doi.org/10.1016/0922-338X\(93\)90151-W](https://doi.org/10.1016/0922-338X(93)90151-W)
- [61] Uslu, H., Inci, I. "(Liquid + liquid) equilibria of the (water + propionic acid + Aliquat 336 + organic solvents) at T = 298.15 K." *Journal of Chemical Thermodynamics*. 39(5), pp. 804–809. 2007. <https://doi.org/10.1016/j.jct.2006.10.001>
- [62] Keshav, A., Chand, S., Wasewar, K. L. "Recovery of propionic acid from aqueous phase by reactive extraction using quaternary amine (Aliquat 336) in various diluents." *Chemical Engineering Journal*. 152(1), pp. 95–102. 2009. <https://doi.org/10.1016/j.cej.2009.03.037>
- [63] Keshav, A., Wasewar, K. L., Chand, S. "Extraction of propionic acid with tri-n-octyl amine in different diluents." *Separation and Purification Technology*. 63(1), pp. 179–183. 2008. <https://doi.org/10.1016/j.seppur.2008.04.012>
- [64] Keshav, A., Wasewar, K. L., Chand, S., Uslu, H. "Effect of binary extractants and modifier-diluents systems on equilibria of propionic acid extraction." *Fluid Phase Equilibria*. 275(1), pp. 21–26. 2009. <https://doi.org/10.1016/j.fluid.2008.09.012>
- [65] Wang, P., Wang, Y., Liu, Y., Shi, H., Su, Z. "Novel in situ product removal technique for simultaneous production of propionic acid and vitamin B12 by expanded bed adsorption bioreactor." *Bioresource Technology*. 104, pp. 652–659. 2012. <https://doi.org/10.1016/j.biortech.2011.10.047>
- [66] Baroi, G. N., Skiadas, I. V., Westermann, P., Gavala, H. N. "Effect of in situ acids removal on mixed glucose and xylose fermentation by *Clostridium tyrobutyricum*." *AMB Express*. 5(1), pp. 67. 2015. <https://doi.org/10.1186/s13568-015-0153-0>
- [67] Kumar, S., Babu, B. V. "A Brief Review on Propionic Acid: A Renewal Energy Source." *Environmental Engineering*. 4, pp. 459–464. 2006.